Week 6 Notebook

July 6, 2015 - July 10, 2015

July 6, 2015

Kayla/Julie

- Prepared samples of 31C6, 31C7, and 31C8 for sequencing
- Performed test transformation with pDC1 and pDC1+GFP competent cells
    - used 14-34C and 14-35C plasmids for the test transformation
    - two controls with just competent cells
- Prepared 5 mL liquid cultures of DH5α, EMG2:Kλ, BclA-YFP, GFP, pDC1, and pDC1+GFP

Chloe/Charlotte

Minipreps of 15-27C and 15-28C cultures grown over the weekend were prepared. Unfortunately, no glycerol stocks were made.

2 cultures of 15-27C were left to incubate at 37℃ until 20 hours of total incubation time was reached. The cultures were then used for a test-plating in order to improve our freeze survival assay protocol. The test plating was performed in the following way:

- 3 10^{-6} dilutions of each culture were prepared via 10-fold dilutions
- 100 ul of each dilution was plated in 2 ways: glass beads or glass “hockey stick”
- Plates went into the incubator and .5 ml aliquots of the cultures went in the freezers at 3:30 pm.

Glycerol stocks of the final two cultures of 15-27C (#7 and #8) were prepared.

The minipreps of 15-27C (#s 1-6) and 15-28C (#s 1 & 2) were digested with EcoRI and PstI using the standard 20 ul protocol and left in the 37℃ water bath overnight.

Dave/Eddie

- Resuspended 11 new AFPs by centrifuging, adding 50 ul water, vortexing, and centrifuging again.
- Ran a digest of all 11 AFPs with 20 ul DNA and 2 ul of each of X and P in 50 ul total reaction and then heat killed them.
- Also digested two minipreps of 15-17C, using the full 30 ul in 50 ul total reaction with S and P.
- Ran a gel to purify the vector with the following wells:
    - Ladder
    - 2-5 15-17C
- Vector was gel extracted and used in ligations.

- Ligations of 11 AFPs were conducted and left to incubate at RT overnight. All ligations were conducted in 2 conditions:
    - AFPs included: Cf, Ap, IA, MP, Mo, Ma, Li, Ng, Ep, Ch, Dc

Ingredient	[AFP] 2	[AFP] 5
Vector (15-17C) (ul)	2	2
Buffer (ul)	2	2
Ligase (ul)	1	1
Insert (ul)	2	5
H2O (ul)	13	10
Total (ul)	20	20

    A no insert control was also created.
- Tested RiAFP in a gradient PCR to determine the efficacy of the two new J23119 primers. Gradient was 12 samples which ranged from 52C to 62C in two sample groups, A and B. Group A used primer 16, or J23119-1 and the 3' biobrick primer. Group B used primer 17, or J23119-2 and the biobrick 3' primer. Based on the gel results, primer 16 is the more effective primer, and it appears to be more effective at higher temperatures. 58C will be used for our colony PCR.

July 7, 2015

Kayla/Julie

- Analyzed sequencing results
    - none of the 31C constructs contained the correct promoter
    - promoter is identical to BBa_J23106
        - BBa_J23117 could have been switched with BBa_J23106 at some point in the cloning process
            - sent both 15-26C minipreps for sequencing
- Started a new biofilm assay plate
    - Rows
        - A-D=LB
        - E-H=M9 Minimal Media
    - Columns
        - 1 and 7=DH5α
        - 2 and 8= EMG2:Kλ
        - 3 and 9= BclA-YFP in EMG2:Kλ
        - 4 and 10=GFP in EMG2:Kλ
        - 5 and 11=pDC1
        - 6 and 12=pDC1+GFP

- Set up a digest of 15-26C miniprep 1
    - 50 µL reaction
        - 10 µL miniprep
        - 2 µL SpeI
        - 2 µL PstI
        - 5 µL Cut Smart Buffer
        - 31 µL H2O
    - digested overnight at 37ºC

Chloe/Charlotte
The test plating from yesterday was investigated; a visual assessment showed that plating using glass beads resulted in greatly decreased variability over plating with a stick.

The digests were removed from the water bath and run on a gel, pictured below.

Minipreps of 15-27C 7 and 15-27C 8 were prepared and test digested with E and P. The resulting gel showed that no insert was present in these cultures.

15-27C 1, 2, and 4 as well as 15-28C 2 were sent for sequencing and transformed, as there were no glycerol stocks of these cultures.

Overnight liquid cultures of 15-17C, 15-21C, 14-34C, and 14-35C were prepared.

Dave/Eddie
- Transformed the 11 AFPs that ligated overnight and plated them for incubation overnight.
- Ran a colony PCR for 11 colonies of ZeAFP (15-19C) at 58C to determine which colonies contain the ZeAFP insert. RiAFP was run as a control.
- Ti AFP and HhAFP were cut using X and P and digested for 2 hours at 37C.
- Ligations of TiAFP and HhAFP, made the same way as yesterday, were made and left to incubate at RT overnight.
- Colony PCR reaction mixture:

Ingredients	1x Master Mix	13x Super Master Mix
PCR MM (ul)	25	305
Primer 16 (ul)	1.25	16.25
Biobrick 3’ Primer (ul)	1.25	16.25
H2O (ul)	22.5	292.5
bacteria	Scraping/edge of colony	Scraping/edge of colony

- Colony PCR program:

	94C	2 min
34 cycles	94C	30 sec
	58C	30 sec
	68C	1 min
	68C	7 min
	10C	Hold

- We ran out of SOC media and so resorted to using LB broth for 10 of the transformations: IAFGP 2 & 5, EpAFP 2 & 5, CfAFP 2 & 5, NgAFP 2, MoAFP 2, ApAFP 5, MaAFP 5, and ChAFP 5.
- The colony PCR showed insert in all of the eleven colonies, so we picked number 2 and number 11 to grow up a liquid culture overnight.

July 8, 2015

Kayla/Julie

- Analyzed sequencing for both 15-26C minipreps
    - no read for miniprep 1 in the forward direction
    - reverse read for miniprep 1 did not contain the correct promoter
    - Miniprep 2 forward read contained the J23117 promoter
        - set up digest of 15-26C miniprep 2 (50 µL total) to assure that we are cloning with the correct promoter
            - digested at 37ºC for 1 hour
            - ran on a 1.0% agarose gel at 90V for 45 minutes
            - gel purified band
- Transformed ligations for TiAFP, HhAFP, BrAFP, GaAFP, and TmAFP
- Ligated 15-5A insert into opened 15-26C vector
    - 20 µL reactions
        - 3 µL 15-26C
        - 3 µL 15-5A (0 µL for control)
        - 2 µL ligase buffer
        - 1 µL ligase
        - 11 µL dH2O (14 µL for control)
        - incubated at room temperature for 1 hour
- Transformed with 2 µL of ligation products

Chloe/Charlotte
We began a freeze assay for 15-17C, 15-21C, 14-34C, and 14-35C, plating 100 ul of the pre-freeze cultures with glass beads and placing .5 ml of each in -20 and -80℃ freezers.

Primers for AFPs were designed to remove start codons, and they were ordered. Primers were numbered iGEM18-37.

The transformation plates of 15-27C 1, 2, and 4, and 15-28C 2 yielded plenty of colonies. 15-27C 1 and 15-28C 2 were sequence-confirmed and five colonies were picked from each plate and overnight liquid cultures were prepared.

Overnight liquid cultures of RFP, GFP, and YFP were prepared for more painting.

Dave/Eddie

- Made glycerol stocks and miniprepped the two colonies of 15-19C. Concentration for the colony 2 couldn't be found as after two attempts the spec was still giving negative numbers, and we wouldn't have enough for sequencing if we did it again. Colony 11 was as follows:

	@260	@280	Concentration
15-19C 11	0.0172	0.0144	284

- Sent away both colonies for sequencing.
- Digested five remaining minpreps of 15-17C with S and P for two hours to get more vector.
- Picked colonies from the plates of the eleven AFPs for colony PCR. They were run on gels in the following order:

1) 32C	6) 33C	11) 36C	16) 39C	21) Control	26) 41C	31) Control
2) 32C	7) 34C	12) 36C	17) 40C*	22) 38C	27) 41C	
3) 32C	8) 35C	13) 36C	18) 39C*	23) 38C	28) 42C	
4) 33C	9) 35C	14) 37C	19) 40C	24) 38C	29) 42C	
5) 33C	10) 35C	15) 39C	20) 40C	25) 41C	30) 42C	

*Mistake made, this reflects the actual plate

- The negative control was accidentally not plated in both gels, instead both samples(of the same control) are on the second plate.

- All bands indistinguishable from negative controls. Different colonies will be picked tomorrow and the process repeated.

July 9, 2015

Kayla/Julie

- Checked transformation plates for TiAFP, HhAFP, BrAFP, GaAFP, TmAFP, and 5A+26C (31C)
    - no colonies grew because plates were runny
    - repeated transformations with new plates
- Stained biofilm plate with crystal violet
    - biofilm formation was observed in EMG2:Kλ, BclA-YFP in EMG2:Kλ, GFP in EMG2:Kλ, pDC1, and pDC1+GFP strains when grown in M9 minimal media

Chloe/Charlotte

The cultures of 15-27C and 15-28C were removed from the shaker and miniprepped.

The plates from the freeze assay were removed from the incubator at 11 am. When they were counted, the results were placed in the table below.

Colonies of Stationary Phase Growth *E. coli* Plates (Before Freeze)							
Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies
14-34C 10-6 (1)	455	14-35C 10-6 (1)	1251	15-21C 10-6 (1)	956	15-17C 10-6 (1)	1093
14-34C 10-6 (2)	855	14-35C 10-6 (2)	673	15-21C 10-6 (2)	370	15-17C 10-6 (2)	593
14-34C 10-6 (3)	163	14-35C 10-6 (3)	890	15-21C 10-6 (3)	735	15-17C 10-6 (3)	165
Average:	491	**Average:**	938	**Average:**	687	**Average:**	617

At 2 pm, the -80 and -20 aliquots from the freeze assay were removed from their respective freezers, thawed, diluted, and plated. The dilutions were performed by a single person using a single pipet and the liquid culture aliquots were vortexed before each dilution.

Dave/Eddie

- Sequencing results indicate colony number 11 has the proper sequence, thus we can conclude that we have ZeAFP! Colony 2 does not seem to have the correct prefix and suffix. Sequencing appears to have gone wrong.

- We ligated 15-20C, 15-22C, 15-23C, 15-43C and 15-44C and gave them to Kayla and Julie to transform.
- Picked new colonies to colony PCR again and ran on a gel with the following wells:

1) 32C	4) 33C	7) 35C	10) 36C	13) 39C	16) 40C
2) 32C	5) 35C	8) 36C	11) 39C	14) 40C	17) + Control
3) 33C	6) 35C	9) 36C	12) 39C	15) 40C	18) - Control

- The bands are still indistinguishable from the negative control, and the positive control looks good. The only possibility is MoAFP, of which one well may have insert. The colony (6) was picked for growth overnight.

July 10, 2015

Kayla/Julie

- Transformed ZeAFP and RiAFP into EMG2:Kλ for biofilm assay
- Quantified biofilm assay
    - resuspended crystal violet in 30% acetic acid
    - transferred solution of crystal violet and acetic acid to new wells on a flat bottom plate
    - measured OD_{595nm} of each well of the flat bottom plate using the plate reader

Chloe/Charlotte

The concentrations of the minipreps prepared yesterday were found using the spectrophotometer. Additionally, the GFP colony was sent for sequencing.

The ligation of CFP+J23119 was transformed, plated, and left to incubate at 37°C overnight.

The colonies on the freeze survival assay plates were counted, and the average number of colonies and percent survival of each plate were calculated.

Colonies of Stationary Phase Growth *E. coli* Plates (After Freeze)							
Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies	Strain & Dilution	Number of Colonies
-20°C							
14-34C 10-6 (1)	235	14-35C 10-6 (1)	687	15-21C 10-6 (1)	124	15-17C 10-6 (1)	1276

14-34C 10-6 (2)	46	14-35C 10-6 (2)	238	15-21C 10-6 (2)	82	15-17C 10-6 (2)	339
14-34C 10-6 (3)	121	14-35C 10-6 (3)	194	15-21C 10-6 (3)	38	15-17C 10-6 (3)	318
Average:	134	**Average:**	373	**Average:**	81.33	**Average:**	644.33
Survival:	0.273	**Survival:**	0.397	**Survival:**	0.118	**Survival:**	1.044
-80℃							
14-34C 10-6 (3)	265	14-35C 10-6 (3)	171	15-21C 10-6 (3)	65	15-17C 10-6 (3)	29
14-34C 10-6 (3)	111	14-35C 10-6 (3)	383	15-21C 10-6 (3)	261	15-17C 10-6 (3)	163
14-34C 10-6 (3)	211	14-35C 10-6 (3)	114	15-21C 10-6 (3)	213	15-17C 10-6 (3)	270
Average:	195.67	**Average:**	222.67	**Average:**	179.67	**Average:**	154
Survival:	0.399	**Survival:**	0.237	**Survival:**	0.262	**Survival:**	0.250

Dave/Eddie

- Miniprepped 15-36C and sent it away for sequencing.
- CIP treated 15-17C for our ligations.
- PCR amplified our stock solutions of 16 AFPs - everything but RiAFP and ZeAFP because they have both been sequence confirmed. A test gel was run with the AFPs in sequential order except for MpAFP, DcAFP and NgAFP which were run at the end.
- The PCR amplified AFPs and the CIP treated 15-17C were all column purified.
- The plated transformations made by Kayla and Julie of five AFPs all showed growth so we ran a colony PCR on three colonies from each AFP to determine if the insert was present with the following wells:

1) 15-20C	4) 15-22C	7) 15-23C	10) 15-43C	13) 15-44C	16) + Control
2) 15-20C	5) 15-22C	8) 15-23C	11) 15-43C	14) 15-44C	17) - Control
3) 15-20C	6) 15-22C	9) 15-23C	12) 15-43C	15) 15-44C	18) - Control

- Two of the colonies looked good (15-23C 2 and 15-43C 2) so those will be picked next week.

July 6, 2015

Kayla/Julie

- Prepared liquid cultures for 3 colonies on 31C transformation plate (26C+purified 5A)
- Prepared 5 mL liquid cultures for ZeAFP and RiAFP in EMG2:Kλ